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Anti-inflammatory factors in human synovial fluid

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Many inflammatory exudates show an anti-inflammatory activity when tested against animal models [1]. In this communication we have examined human rheumatoid synovial fluid since this is an exudate produced by a human inflammatory disease rather than by an irritant in an animal model.

Method. Human rheumatoid synovial fluid (sera-positive R.A.) was aspirated from patients at the Royal National Hospital for Rheumatic Diseases (Bath, U.K.). After aspiration the samples were centrifuged to remove cells (8000 *g* for 25 min at 4°) and stored at −30° until used. Carrageenan-induced oedema in the rat paw [2] was used to assess anti-inflammatory activity. The rats were injected i.p. with 1 ml of pooled synovial fluid 1 hr before the injection of carrageenan (0.05 ml) (2% w/v in 0.9% saline) into the hind foot. Controls were treated with saline. The oedema in the injected foot was measured 6 hr after the injection of the carrageenan by a plethysomographic method [3]. The irritancy [4] of solutions and fluids tested for anti-inflammatory activity was determined by injecting

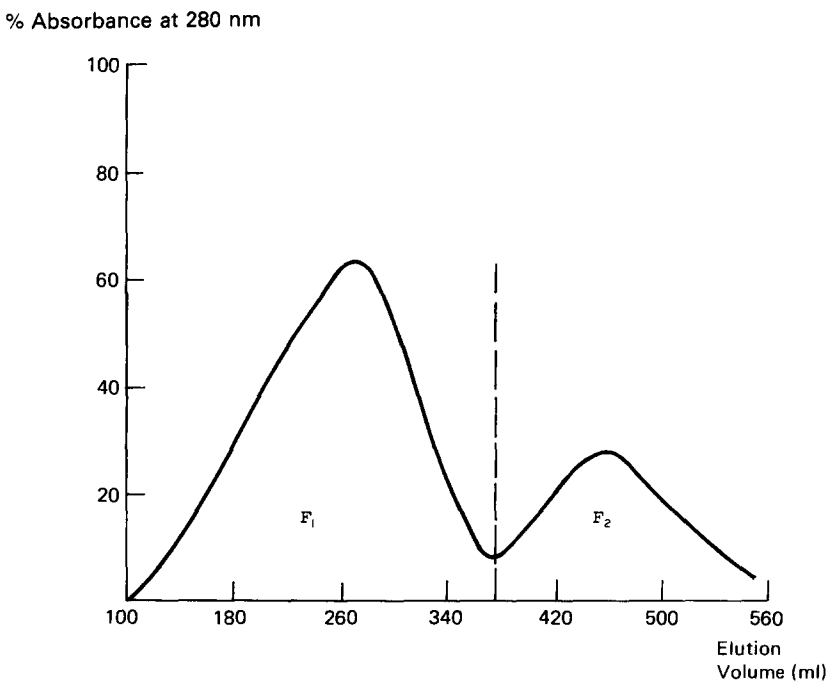

Fig. 1. The protein elution pattern of human rheumatic synovial fluid through a Sephadex G-150 column in 0.1 M phosphate buffer (pH 7.4). The absorbance at 280 nm of the eluted proteins was measured. Fractions pooled are indicated by F_1 and F_2.

0.05 ml into the rat's hind paw and determining the oedema produced 6 hr after the injection.

Pooled fluid (5 ml) was applied to a 100 × 2.5 cm column packed with Sephadex G-150 and eluted with 0.1 M phosphate buffer (pH 7.4). The eluate was monitored at 280 nm using a Uvicord II flow through cell and 10-ml fractions collected in a Ultrorac 7000 fraction collector (LKB Instruments, U.K.). The fractions were pooled according to the protein pattern, dialysed against distilled water for 24 hr and finally freeze dried and stored at −30°. The protein fractions were dissolved in saline (100 mg/ml) and tested for anti-inflammatory activity in the carrageenan rat model by injecting 1 ml i.p. 1 hr before the injection of carrageenan. They were also tested for irritancy [4].

Since anti-inflammatory proteins may stabilize membranes [5] the effect of fluid and protein fractions was tested on guinea-pig macrophages. These were obtained by injecting guinea-pigs i.p. with 100 ml of 0.9% (w/v) saline and repeating the treatment 12 hr later immediately before draining the peritoneal cavity. The macrophages (mainly monocytes) were centrifuged down (1000 *g* for 10 min), washed with saline at 4° and suspended in 0.1 M phosphate buffer (pH 7.4) at a protein concentration of 7 mg/ml [6]. Test solutions (1-ml) were incubated with 2 ml of macrophage suspension for 90 min at 37° in a shaking water bath. Freeze dried pooled synovial fluid and its isolated proteins were tested over the concentration range 1–100 mg/ml. After incubation the suspensions were centrifuged at 1000 *g* for 10 min and the supernatants assayed for acid phosphatase activity [7]. Viability counts on the cells were made at the start, and completion of the experiment by trypan blue exclusion. Bovine serum albumin and human albumin were used as protein controls.

Results and discussion. The proteins in synovial fluid separated on Sephadex G-150 into two clear fractions. These fractions were F_1 (high mol. wt fraction) and a smaller F_2 (< 6300 mol. wt) fraction (Fig. 1).

Both fractions were anti-inflammatory (Fig. 2) ($P < 0.05$) but synovial fluid was not. Synovial fluid and fractions F_1 and F_2 were irritant to the rat paw but synovial fluid produced the largest oedema (0.97 ml) compared to fraction F_1 (0.48 ml), fraction F_2 (0.37 ml) and saline (0.05 ml). Irritancy did not parallel anti-inflammatory activity since the most irritant substance, synovial fluid, was not anti-inflammatory.

Synovial fluid was lytic to macrophages resulting in a massive release of acid phosphatase (Fig. 3) but fractions F_1 and F_2 were very effective in stabilizing macrophages and the effect was concentration-dependent. Previous work [5] has shown that proteins in synovial fluids can stabilize liver lysosomes and two proteins in the fluids were found to be associated with the lysosomes. [5].

In summary human rheumatoid fluid contains non-dialysable proteins which possess anti-inflammatory activity and proteins in the fluids can also inhibit the release of hydrolytic enzymes from leucocytes.

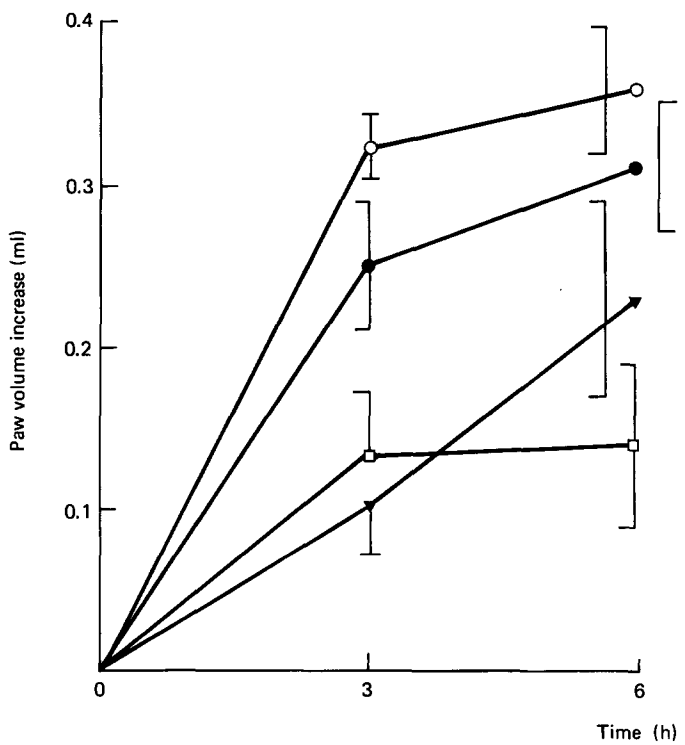

Fig. 2. Increase in foot volume of rats injected with carrageenan and treated with human rheumatic synovial fluid (●——●), fraction F_1 (□——□), fraction F_2 (▼——▼) and saline alone (○——○). Each result represents the mean of six animals ± S.E.M.

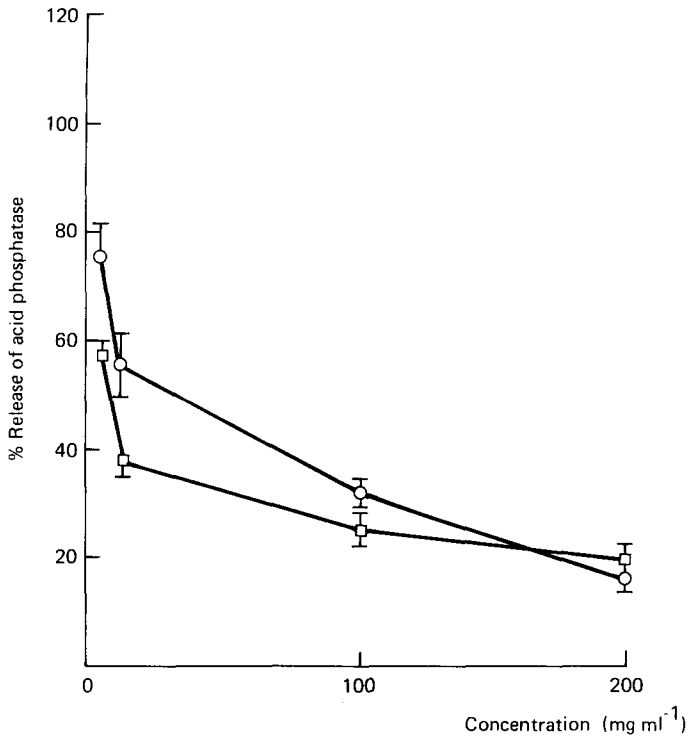

Fig. 3. The effect of fractions F_1 (○—○) and F_2 (□—□) on the release of acid phosphatase from macrophages. The % release of acid phosphatase was calculated as the percentage of final minus initial values for the controls where protein was omitted. Therefore values > 100 are lytic and values < 100 are stabilizing. The synovial fluid and fractions were also incubated for 90 min without cells and their acid phosphatase values subtracted from values obtained when cells were present. Viability counts showed that 98.2% of the cells were viable at the start of the experiment and over 98% were still viable at the end of the experiment for all samples examined. Bovine serum albumin and human albumin substituted for the protein fractions had no effect on the release of acid phosphatase from the macrophages. The corresponding data for freeze dried synovial fluid (not plotted) were: 1 mg/ml, 96 ± 6%; 10 mg/ml, 100 ± 10%; 100 mg/ml, 170 ± 9%; and 200 mg/ml, 250 ± 21%. Each result is the mean of four experiments ± S.E.M. The acid phosphatase activity of the pooled synovial fluid used was 0.6 mg *p*-nitrophenol/ml.

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